Genomic Profiling of Advanced-Stage, Metaplastic Breast Carcinoma by Next-Generation Sequencing Reveals Frequent, Targetable Genomic Abnormalities and Potential New Treatment Options

Jeffrey S. Ross, MD; Sunil Badve, MD; Kai Wang, PhD; Christine E. Sheehan, MS; Ann B. Boguniewicz, MD; Geoff A. Otto, PhD; Roman Yelensky, PhD; Doron Lipson, PhD; Siraj Ali, MD, PhD; Deborah Morosini, MD; Juliann Chliemlecki, PhD; Julia A. Elvin, MD, PhD; Vincent A. Miller, MD; Philip J. Stephens, PhD

• ***Context.***—**Metastatic metaplastic breast carcinoma (MPBC) is an uncommon, but aggressive, tumor resistant to conventional chemotherapy.**

Objective.—**To learn whether next-generation sequencing could identify potential targets of therapy for patients with relapsed and metastatic MPBC.**

Design.—**Hybridization capture of 3769 exons from 236 cancer-related genes and 47 introns of 19 genes commonly rearranged in cancer was applied to a minimum of 50 ng of DNA extracted from 20 MPBC formalin-fixed, paraffin-embedded specimens and sequenced to high uniform coverage.**

Results.—**The 20 patients with MPBC had a median age of 62 years (range, 42–86 years). There were 9 squamous (45%), 9 chondroid (45%), and 2 spindle cell (10%) MPBCs, all of which were high grade. Ninety-three genomic alterations were identified, (range, 1–11) with 19 of the 20 cases (95%) harboring an alteration that could potentially lead to a targeted treatment option. The most-common alterations were in *TP53* (n = 69; 75%), *PIK3CA* (n = 37; 40%), *MYC* (n = 28; 30%), *MLL2* (n = 28; 30%), *PTEN* (n = 23; 25%), *CDKN2A/B* (n = 19; 20%), *CCND3* (n = 14; 15%), *CCNE1* (n = 9; 10%), *EGFR* (n = 9; 10%), and *KDM6A* (n = 9; 10%); *AKT3*, *CCND1*, *CCND2*, *CDK4*, *FBXW7*, *FGFR1*, *HRAS*, *NF1*, *PIK3R1*, and *SRC* were each altered in a single case. All 16 MPBCs (100%) that were negative for ERBB2 (HER2) overexpression by immunohistochemistry and/or *ERBB2* (*HER2*) amplification by fluorescence in situ hybridization were also uniformly (100%) negative for *ERBB2* amplification by next-generation sequencing–based copy-number assessment.**

Conclusions.—**Our results indicate that genomic profiling using next-generation sequencing can identify clinically meaningful alterations that have the potential to guide targeted treatment decisions in most patients with metastatic MPBC.**

(*Arch Pathol Lab Med.* 2015;139:642–649; doi: 10.5858/arpa.2014-0200-OA)

Metaplastic carcinoma of the breast (MPBC) is an uncommon, malignant tumor with a variable histologic appearance that differs substantially from classic invasive ductal adenocarcinoma and invasive lobular adenocarcinoma.[1–5] Metaplastic carcinoma of the breast typically presents at a more-advanced stage than either invasive ductal or lobular adenocarcinoma cases and, in general, has a worse prognosis.[6,7] On routine biomarker testing for estrogen receptor (ER) and/or progesterone receptor (PR) status, most MPBCs are negative.[8] Similarly, virtually all MPBC cases are negative for HER2 overexpression and amplification by either immunohistochemistry (IHC) and/or fluorescence in situ hybridization.[8,9] These findings combined with messenger RNA (mRNA) profiling studies indicate that most MPBCs are so-called triple-negative breast cancers (TNBCs) that cluster with the basaloid-phenotype cancers in the breast cancer molecular-portraits system.[10–12] However, although the TNBC and basaloid types of breast cancer are classically associated with responsiveness to cytotoxic chemotherapy,[13,14] metastatic MPBC is generally regarded as a chemoresistant, highly aggressive form of the disease.[15,16] Given the poor prognosis and reduced response to treatment for MPBC, this study was performed to evaluate potential, targeted treatment opportunities for patients with MPBC.

Accepted for publication June 10, 2014.

From the Department of Pathology and Laboratory Medicine, Albany Medical College, Albany, New York (Drs Ross and Boguniewicz and Ms Sheehan); Research and Development, Foundation Medicine, Inc, Cambridge, Massachusetts (Drs Ross, Wang, Otto, Yelensky, Lipson, Ali, Morosini, Chliemlecki, Elvin, Miller, and Stephens); and the Department of Pathology, University of Indiana School of Medicine, Indianapolis (Dr Badve).

Drs Ross, Wang, Otto, Yelensky, Lipson, Ali, Morosini, Chliemlecki, Elvin, Miller, and Stephens are all employees of Foundation Medicine, Inc, Cambridge, Massachusetts. Dr Ross has stock ownership and receives research support from Foundation Medicine. Drs Wang, Otto, Yelensky, Lipson, Morosini, Chliemlecki, Elvin, Miller, and Stephens have stock ownership in Foundation Medicine. The other authors have no relevant financial interest in the products or companies described in this article.

Reprints: Jeffrey S. Ross, MD, Department of Pathology and Laboratory Medicine, Albany Medical College, 47 New Scotland Ave, Albany, NY 12208 (e-mail: rossj@mail.amc.edu).

Table 1. Genomic Alterations in 20 Cases of Advanced-Stage Metaplastic Breast Carcinoma

Case No.	Percentage of Tumor by Microscopy/Tumor Purity[a]	Coverage Depth, ×	Age, y	Sample Used for NGS	Subtype	Tumor Grade	Tumor Stage	Clinical ER/PR/HER2 Status	Genomic Alterations, Mutant Allele Frequency (%); Copy No.
B01	70/30	711	62	Breast	Squamous	3	4	ER$^-$/PR$^-$/HER2$^-$	*PTEN* loss; 0 *RB1* splice site 607$^+$2T>A (19) TP53 Y220S (26) *CREBBP* K1086* (18)
B02	80/30	749	63	Lymph node	Squamous	3	3		*PTEN* R14fs*10 (24) *TP53* V173fs*7 (33) *TNFRSF*14 loss; 0
B03	60/20	906	49	Breast	Squamous	3	2	ER$^+$/PR$^+$/HER2$^-$	*PIK3CA* H1047R (4) *TP53* K320* (5)
B04	60/70	877	73	Breast	Spindle	3	3		*MCL1* amplification; 7 *ARID1A* R1722* (4) *NFKBIA* amplification; 6 *PTPN11* S502L (2)
B05	70/40	926	71	Breast	Spindle	3	4	ER$^-$/PR$^-$/HER2$^-$	*PIK3R1* Y580fs*19 (42) *CCND2* amplification; 24 *CDKN2A* loss; 0 *FGF23* amplification; 24
B06	60/84	1123	53	Lung	Chondroid	3	4	ER$^-$/PR$^-$/HER2$^-$	*FANCA* C1410fs*6 (12) *MYC* amplification; 8
B07	30/58	1234	61	Chest wall	Chondroid	3	4	ER$^-$/PR$^-$/HER2$^-$	*CDK4* amplification;15 *MYC* amplification; 9 *TP53* R248Q (61)
B08	80/85	1214	67	Breast	Chondroid	3	4	ER$^-$/PR$^-$/HER2$^-$	*CCND1* amplification; 7 *TP53* C277F (84) LRP1B S229* (41) *MLL2* V1554fs*48 (40) *FGF19* amplification; 7 *FGF3* amplification; 7 *FGF4* amplification; 7
B09	70/80	1073	59	Brain	Squamous	3	4	ER$^-$/PR$^-$/HER2$^-$	*PTEN* K327fs*16 (63) *TP53* N131del (75) *KDM6A* Q692fs*37 (56) *RB1* splice site 1421$^+$1G>C (64)
B10	80/40	1020	57	Chest wall	Chondroid	3	4	ER$^-$/PR$^-$/HER2$^-$	*CDKN2A/B* loss; 0 *HRAS* Q61H (25) *MLL* R2075H (23) *MSH2* loss; 0 *TNFAIP3* V377M (24) *MLL2* P2354fs*30 (23)
B11	50/49	472	53	Breast	Squamous	3	4		*EGFR* amplification; 7 *PTEN* loss; 0 *CCND3* amplification; 8 *MYC* amplification; 7 *TP53* R273C (66) *MYST3* amplification; 7

Table 1. Continued

Case No.	Percentage of Tumor by Microscopy/Tumor Purity[a]	Coverage Depth, ×	Age, y	Sample Used for NGS	Subtype	Tumor Grade	Tumor Stage	Clinical ER/PR/HER2 Status	Genomic Alterations, Mutant Allele Frequency (%); Copy No.
B12	70/76	735	66	Breast	Chondroid	3	4	ER$^-$/PR$^+$/HER2$^-$	*PIK3CA* H1047R (45) *PTEN* loss; 0 *ATR* R177Q (62) *CDKN2A/B* loss; 0 *TP53* E285K (47) *PRKDC* V3312M (46)
B13	60/39	934	86	Breast	Squamous	3	4	ER$^-$/PR$^-$/HER2$^-$	*PIK3CA* H1047R (8)
B14	70/20	870	69	Breast	Squamous	3	4	ER$^-$/PR$^-$/HER2$^-$	*TP53* P64fs*85 (33) *RB1* D156fs*19 (17) *MED12* G44D (14)
B15	80/18	684	64	Lung	Chondroid	3	4		*PIK3CA* E545K (48) *CCND3* amplification; 7 *CCNE1* amplification; 8 *MYC* amplification; 13 *TP53* S94* (58) *MLL2* L4426fs*6 (10)
B16	80/60	608	42	Breast	Chondroid	3	4	ER$^-$/PR$^-$/HER2$^-$	*PIK3CA* E542K (59) *TP53* R273C (78) *MLL2* Q3499* (77)
B17	40/60	607	60	Breast	Chondroid	3	4	ER$^-$/PR$^-$/HER2$^-$	*EGFR* amplification; 30 *PIK3CA* H1047R (76) *PI3KCA* amplification; 16) *CDKN2A/B* loss; 0) SOX2 amplification; 7) *TP53* splice site 814_919$^+$192del (34)
B18	70/30	598	43	Breast	Squamous	3	4	ER$^-$/PR$^-$/HER2$^-$	*AKT3* amplification; 8) *FBXW7* Q508* (28) NF1 V891fs*10 (21) *TP53* splice site 559$^+$1G>*T* (45) *KDM6A* N1130fs*8 (19) *LRP1B* deletion, exons 4–21 (not determined) *PRKDC P*1159S SF3B1 K666T (27) *MLL2* F2739fs*18 (27) *PALB2* W1038* (21)
B19	70/30	593	71	Breast	Squamous	3	4	ER$^-$/PR$^-$/HER2$^-$	*PIK3CA* E545K (45) *MCL1* amplification; 7 *CCNE1* amplification; 9 *TP53* S94* (59) *MYC* amplification; 7 *CCND3* amplification; 7 *MLL2* L4426fs*6 (8)

Table 1. Continued

Case No.	Percentage of Tumor by Microscopy/Tumor Purity[a]	Coverage Depth, ×	Age, y	Sample Used for NGS	Subtype	Tumor Grade	Tumor Stage	Clinical ER/PR/HER2 Status	Genomic Alterations, Mutant Allele Frequency (%); Copy No.
B20	30/58	733		Breast	Chondroid	3	4	ER−/PR−/HER2−	*FGFR1* amplification; 13 *SRC* amplification; 9 *MYC* amplification; 8 *TP53* R273C (84) *LRP1B* splice (27) *C17orf39* amplification; 12

Abbreviation: NGS, next generation sequencing.

[a] Calculated estimate of the percentage of DNA derived from tumor DNA based on aneuploidy.

MATERIALS AND METHODS

Hybridization capture of 3769 exons from 236 cancer-related genes and 47 introns of 19 genes commonly rearranged in cancer was applied to a minimum of 50 ng of DNA extracted from 20 formalin-fixed, paraffin-embedded cases of relapsed and metastatic MPBC that had previously been treated with systemic therapies.[17] There were no early stage or untreated MPBC cases included in this study. The 20 cases represented all (100%) of the MPBC samples received at Foundation Medicine, Inc (Cambridge, Massachusetts), for next-generation sequencing between January 1, 2013, and February 1, 2014. The 20 MPBC samples were sequenced to high, uniform coverage (average ×833, with >99% of exons covered at greater than ×100). All MPBC cases were reviewed by 2 pathologists (J.S.R. and S.B.) and were subdivided into histologic subtypes: predominantly spindle cell (sarcomatoid), predominantly squamous, predominantly chondroid, and mixed. Formalin-fixed, paraffin-embedded specimens were sequenced to high (average ×833), uniform coverage, as previously described.[17] Genomic alterations (base substitutions, small insertions/deletions [indels], select rearrangements, and copy-number alterations) were determined. *Potentially actionable alterations* were defined as those linked to anticancer drugs on the market or in registered clinical trials. Local site permissions to use clinical samples and their accompanying medical records and pathology reports were used for this study. No additional research was performed on the tumor samples beyond the DNA sequencing.

RESULTS

The 20 patients with MPBC had a median age of 62 years (range, 42–86 years). There were 9 predominantly squamous (45%), 9 predominantly chondroid (45%), and 2 predominantly spindle cell (sarcomatoid) (10%) MPBCs, all of which were originally diagnosed as high-grade tumors (Table 1). All MPBCs (20 of 20; 100%) had pure MPBC histology, and none of the cases (0 of 20; 0%) in this series had foci of either classic invasive ductal carcinoma or invasive lobular carcinoma admixed with the metaplastic carcinoma areas. All of the MPBCs (20 of 20; 100%) were advanced stage: 1 MPBC (5%) was stage II, 2 (10%) were stage III, and 17 (85%) were stage IV. Because this study included only patients with relapsed and metastatic disease, there were no well-differentiated or stage-I tumors included in this patient cohort. Of the 16 cases (80%) with available biomarker results, 6% (n = 1) were ER^+, 12% (n = 2) were PR^+, and 100% (n = 16) were $HER2^-$ by either IHC or fluorescence in situ hybridization testing. Thus, 87% of the MPBCs were TNBC and 13% were either ER^+ or PR^+ positive. The targeted next-generation sequencing assay used in this study identified 93 genomic alterations in the 20 MPBC, with at least one alteration identified in all cases (mean [SD], 4.65 [2.08] per tumor). Nineteen (95%) of the 20 MPBC cases harbored 36 alterations that could potentially lead to a targeted therapeutic treatment option (mean [SD], 1.8 [1.08] per tumor).[18,19] The most-common, biologically relevant alterations were alterations in *TP53* (n = 15; 75%), *MYC* (n = 6; 30%), *MLL2* (n = 6; 30%), and *KDM6A* (n = 2; 10%). The most-common, potentially targetable alterations were mutations, amplifications, and homozygous deletions of *PIK3CA* (n = 8; 40%), *PTEN* (n = 5; 25%), *CDKN2A/B* (n = 4; 20%), *CCND3* (n = 3; 15%), *CCNE1* (n = 2; 10%), and *EGFR* (n = 2; 10%), with *AKT3*, *CCND1*, *CCND2*, *CDK4*, *FBXW7*, *FGFR1*, *HRAS*, *NF1*, *PIK3R1*, and *SRC* altered in a single case (Figure 1; Table 1). The actionable alterations discovered in the MPBC have potential for a variety of targeted therapies, including inhibitors of mTOR, cyclin-dependent kinase inhibitors,

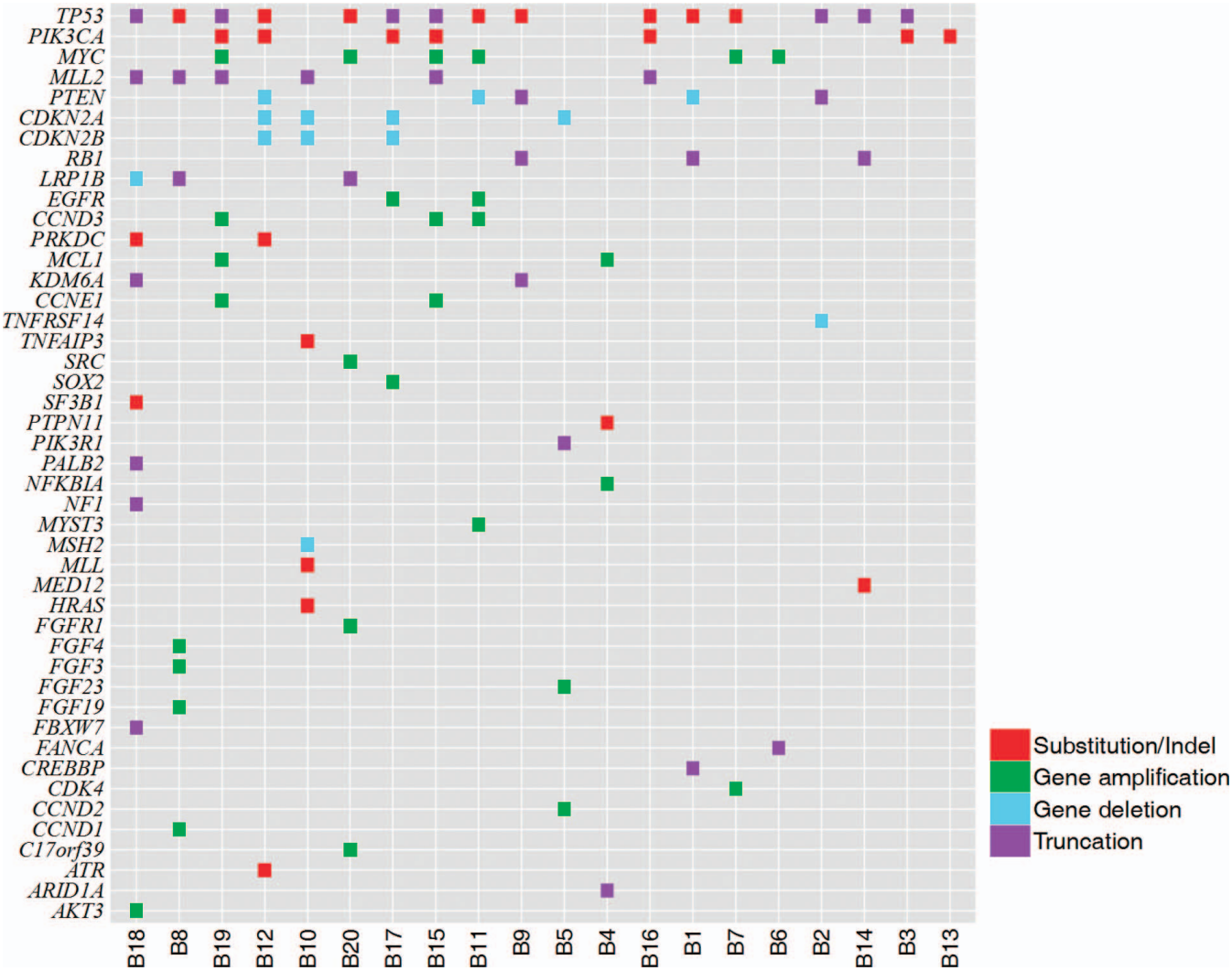

Figure 1. *Tile plot of genomic alterations detected by genomic profiling of 20 cases of advanced-stage metaplastic breast carcinoma.*

MEK, EGFR, FGFR1, and SRC. The results of routine HER2 testing were available for 16 patients (80%) with MPBC and all (100%) were negative for HER2 overexpression, as determined by IHC and/or negative for HER2 amplification as determined by fluorescence in situ hybridization at outside laboratories. All 16 (100%) of those cases were also negative for *ERBB2* copy-number gain (amplification) by the next-generation sequencing assay.

COMMENT

Molecular studies of MPBC to date have predominantly used IHC, fluorescence in situ hybridization, and mRNA transcriptional profiling techniques and have demonstrated that MPBC clusters with the TNBC/basaloid phenotypes.[20–22] Metaplastic carcinomas of the breast have further been associated with the so-called claudin-low subtype of the basaloid phenotype in the molecular-portraits system.[23] In addition, mRNA profiling has linked the various histologic appearances of MPBC to various expression of a diverse group of genes.[24–25] Studies[26] of *TP53* have demonstrated that *TP53* mutation is present in both the epithelial and mesenchymal components in MPBC with mixed histologies at a similar frequency (~75%). In the current study, the 20 MPBCs showed a histologically uniform pattern in that none of the tumors featured a mixture of differentiated invasive ductal adenocarcinoma with metaplastic spindle cell (sarcomatoid), squamous, or chondroid foci. Although the numbers of cases in each category is small, we identified no specific pattern of altered cancer genes that were associated with the sarcomatoid (spindle cell), squamous, or chondroid histologic subtypes. Similarly, when the MPBC cases are categorized into pure epithelial (0 cases; 0%), pure mesenchymal (7 cases; 35%), and mixed epithelial and mesenchymal (13 cases; 65%) groups, no significant differences in the genomic alterations between groups was observed. Instead, similar to prior profiling studies, we identified a heterogeneous combination of alterations, yielding a relatively unique mutation profile for each tumor despite apparent histologic similarities. Additionally, the current study also confirms 2 noteworthy findings from previously published MPBC transcriptional profiling studies: *EGFR* amplification in the absence of *EGFR* mutations[27–28] and alterations in the Wnt signaling pathway without mutations in *CTNNB1*.[29–31]

Given the histologic picture of MPBC with various types of mesenchymal differentiation, including the spindle cell

Table 2. Significant Targetable Genomic Alterations Discovered by Next-Generation Sequencing Assessment of 20 Cases of Advanced-Stage Metaplastic Breast Carcinoma (MPBC)

Gene (%)	Genomic Alterations				Total Targetable Genomic Alterations, No.	Potential, Targeted Therapeutic
	Loss/Homozygous Deletion, No.	Base Substitution, No.	Truncation, No.	Amplification, No.		
PIK3CA (40)		8			8	Everolimus Temsirolimus
PTEN (25)	3		2		5	Everolimus Temsirolimus
CDKN2A/B (20)	4				4	CDK 4/6 inhibitors
CCND3 (15)				3	3	Nutlins
CCNE1 (10)				2	2	Nutlins
EGFR (10)				2	2	Erlotinib Afatinib Gefitinib
HRAS (5)		1			1	Trametinib
AKT3 (5)				1	1	Everolimus Temsirolimus
CCND1 (5)				1	1	Nutlins
CCND2, (5)				1	1	Nulins
CDK4 (5)				1	1	CDK 4/6 inhibitors
FBXW7 (5)			1		1	Everolimus Temsirolimus
FGFR1 (5)				1	1	Pazopanib Ponatinib Regorafenib
NF1 (5)			1		1	Everolimus Temsirolimus
PI3KR1 (5)			1		1	Everolimus Temsirolimus
SRC (5)				1	1	Bosutinib Dasatinib

(sarcomatoid) and chondroid variants of the disease, there has been significant interest in studying genes and biologic pathways associated with the epithelial to mesenchymal transition and the cancer stem cell hypothesis[32–37] in MPBC. Previous studies using IHC or mRNA profiling of MPBC have identified altered expression levels of epithelial to mesenchymal transition genes or directly related pathways. In particular, altered expression of *SNAIL*, a transcriptional repressor of E-cadherin (*CDH1*), has been proposed as an important epithelial-mesenchymal transition pathway gene associated with recurrence and metastasis in the disease, especially in the chondroid variant of MPBC.[38–40] However, in this study, sequence alterations or copy-number gains or losses of these epithelial-mesenchymal transition-associated genes were not identified. In particular, no mutations or homozygous deletions of *CDH1* were identified in this study.

The disease-free and overall survival results for patients with MPBC is significantly shorter than that for non-MPBC treated with cytotoxic agents in either the metastatic or the neoadjuvant settings.[12–14] Nineteen of the 20 MPBC cases (95%) in this series harbored 36 alterations that could potentially lead to a targeted, therapeutic treatment option (mean [SD], 1.8 [1.06] per tumor). This result is similar to a series[41] of 273 routine (non-MPBC) breast cancers evaluated using the same assay, where 246 (90%) harbored at least one potentially actionable alteration. The clinically meaningful alterations that could conceivably guide targeted treatment decisions were identified in 19 of 20 (95%) of the patients in multiple, biologic pathways (Table 2). Cell-cycle alterations were common, including homozygous deletion of *CDKN2A* in 4 (20%) and amplifications of *CCND3*, *CCNE1*, *CCND1*, *CCND2*, and *CDK4* in 8 (40%) of the MPBC cases, a subset of which may indicate the potential for use of cell-cycle inhibitors. An example is case B07, a chest wall relapse of a MPBC in a 61-year-old woman whose tumor demonstrated amplification of *CDK4* (17 copies), in addition to amplification of *MYC* (9 copies) and the R248Q base substitution in *TP53* (Figure 2, A through C). *CDK4* amplification has been reported[42] in 1.5% to 15% of breast carcinomas and expression has been correlated with amplification. Recent results[43–46] focused on targeting cell-cycle regulatory genes in clinical trials for patients with cancer, and alterations in those cell growth regulatory pathways are showing significant promise.

PIK3CA mutations are important genomic alterations in the pathogenesis and progression of breast cancer.[47–49] *PIK3CA* mutations were identified in 8 (40%) of the patients with MPBC in the current study (Figure 3, A and B), which is consistent with a previous study[35] that demonstrated *PIK3CA* mutations in 9 of 19 MPBCs (48%). The *PIK3CA* mutation frequency in MPBC thus appears to be greater than the 25% frequency listed for all types of breast cancer[50] and for TNBCs as a group.[51] *PIK3CA* mutations have been linked to improved outcomes in breast cancer.[52] The PI3K pathway is now widely considered a target of therapy for cancer in general[53] and for breast cancer, in particular.[54] Moreover, activating mutations in *PIK3CA* predict sensitivity to inhibitors of PI3K or its downstream signaling pathway (the PI3K/AKT/mTOR pathway).[55] The mTOR inhibitor everolimus has been approved for use in hormone receptor–positive, HER2$^-$, advanced breast cancer, in combination with exemestane, and clinical trials with other mTOR inhibitors continue in breast cancer.[56] PTEN (phos-

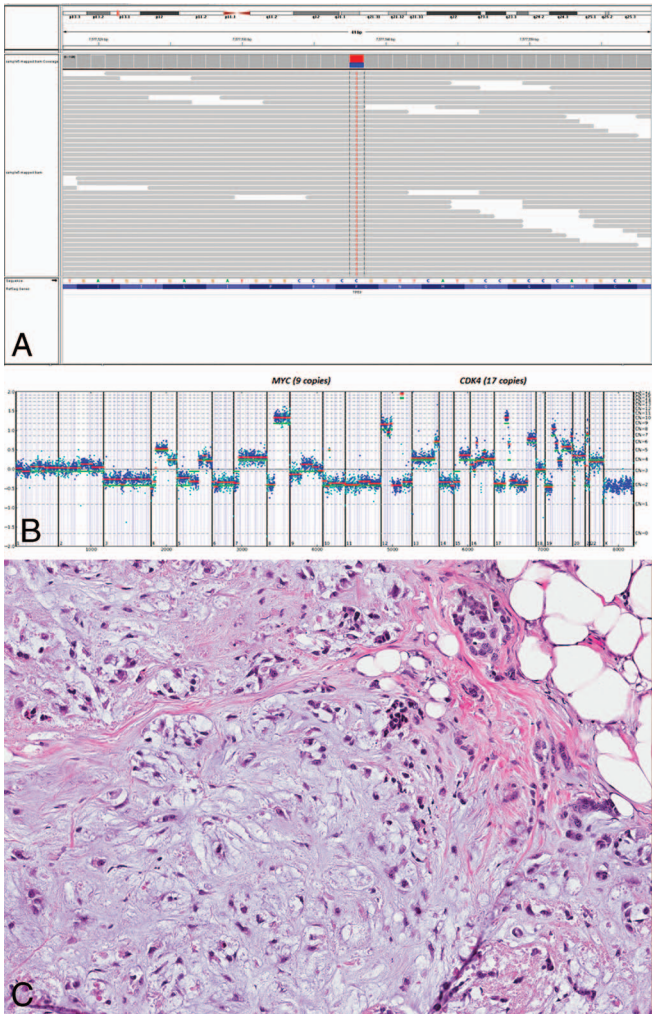

Figure 2. *Chest wall recurrence of a stage-IV chondroid metaplastic breast carcinoma in a 61-year-old woman (case B07). A, An* aTP53 *R248Q base substitution mutation. B, The copy-number plot showing amplifications of the* CDK4 *(16 copies) and* MYC *(9 copies) genes. C, A hematoxylin-eosin–stained slide of the recurrent tumor showing high tumor grade and chondroid appearance (original magnification ×10).*

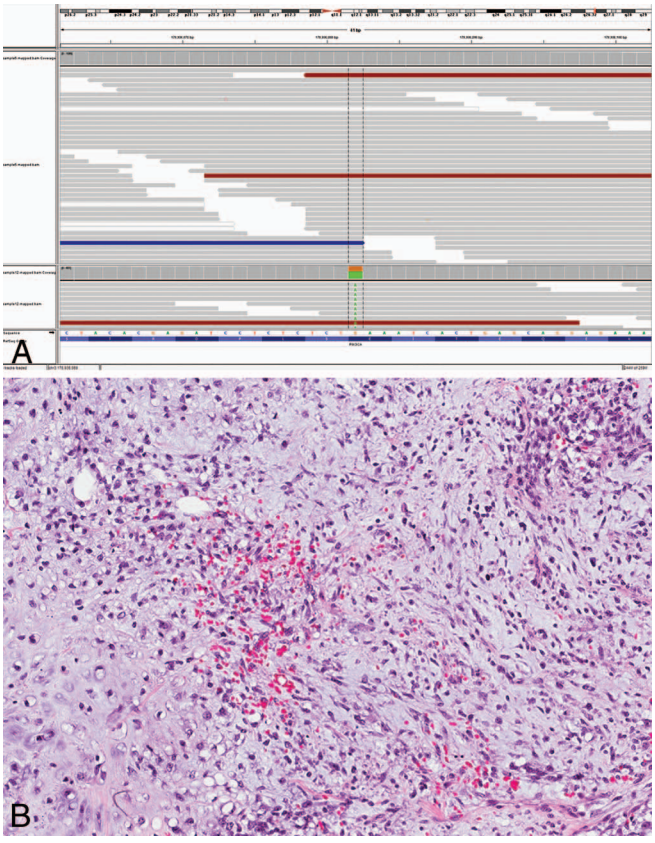

Figure 3. *A high-grade, stage-IV, triple-negative metaplastic breast carcinoma with chondroid differentiation in a 60-year-old woman (case B16). A, An E542K base substitution in the* PIK3CA *gene. Genomic profiling also revealed* TP53 *R273C and* MLL2 *Q3499* mutations. B, A hematoxylin-eosin–stained section of the primary metaplastic breast carcinoma showing chondroid differentiation (original magnification ×10).*

phatase and tensin homolog) functions as a tumor suppressor by negatively regulating the PI3K/Akt/mTOR pathway.[57] Mutations in *PTEN* have been reported[50] in 5% of breast cancers but have not been previously analyzed, to our knowledge, specifically in metaplastic breast carcinoma. In this study, 5 of the 20 MPBC cases (25%) harbored *PTEN* alterations, including 3 homozygous deletions and 2 truncating mutations, which could potentially benefit from PI3K inhibitors in at least a subset of these typically difficult-to-treat breast cancers.

In summary, MPBC is a rare, aggressive subtype of breast cancer, which remains a significant clinical problem given the high propensity to present at advanced stages and progress rapidly in a chemoresistant fashion. This study identified at least one clinically meaningful alteration that could potentially lead to a targeted treatment option in most patients. However, the future effect of the cost of the next-generation sequencing testing and potential use of targeting agents for metastatic MPBC must be weighed against the potential for increasing disease-free and overall survival in this disease. Moreover, the further development of personalized oncology for MPBC and all types of breast cancer will require the continued development of mechanisms driven clinical trials, such as the emerging umbrella and basket trials, which are based on the use of biomarkers to select patients for both single-agent and multiagent, novel treatments. Given the poor prognosis and limited treatment options for patients with metastatic MPBC, genomic profiling using next-generation sequencing has the potential to identify new treatment paradigms and to fulfill an unmet clinical need in this disease.

References

1. Luini A, Aguilar M, Gatti G, et al. Metaplastic carcinoma of the breast, an unusual disease with worse prognosis: the experience of the European Institute of Oncology and review of the literature. *Breast Cancer Res Treat*. 2007;101(3):349–353.

2. Yamaguchi R, Horii R, Maeda I, et al. Clinicopathologic study of 53 metaplastic breast carcinomas: their elements and prognostic implications. *Hum Pathol*. 2010;41(5):679–685.

3. Rungta S, Kleer CG. Metaplastic carcinomas of the breast: diagnostic challenges and new translational insights. *Arch Pathol Lab Med*. 2012;136(8):896–900.

4. Badve S, Dabbs DJ, Schnitt SJ, et al. Basal-like and triple-negative breast cancers: a critical review with an emphasis on the implications for pathologists and oncologists. *Mod Pathol*. 2011;24(2):157–167.

5. Lee H, Jung SY, Ro JY, et al. Metaplastic breast cancer: clinicopathological features and its prognosis. *J Clin Pathol*. 2012;65(5):441–416.

6. Jung SY, Kim HY, Nam BH, et al. Worse prognosis of metaplastic breast cancer patients than other patients with triple-negative breast cancer. *Breast Cancer Res Treat*. 2000;120(3):627–637.

7. Rayson D, Adjei AA, Suman VJ, Wold LE, Ingle JN. Metaplastic breast cancer: prognosis and response to systemic therapy. *Ann Oncol*. 1999;10(4):413–419.
8. Tse GM, Tan PH, Putti TC, Lui PC, Chaiwun B, Law BK. Metaplastic carcinoma of the breast: a clinicopathological review. *J Clin Pathol*. 2006(10);59:1079–1083.
9. Reis-Filho JS, Milanezi F, Steele D, et al. Metaplastic breast carcinomas are basal-like tumours. *Histopathology*. 2006;49(1):10–21.
10. Perou CM, Sorlie T, Eisen MB, et al. Molecular portraits of human breast tumours. *Nature*. 2000;406(6797):747–752.
11. Okada N, Hasebe T, Iwasaki M, et al. Metaplastic carcinoma of the breast. *Hum Pathol*. 2010;41(7):960–970.
12. Bae SY, Lee SK, Koo MY, et al. The prognoses of metaplastic breast cancer patients compared to those of triple-negative breast cancer patients. *Breast Cancer Res Treat*. 2011;126(2):471–478.
13. Nagao T, Kinoshita T, Hojo T, Tsuda H, Tamura K, Fujiwara Y. The differences in the histological types of breast cancer and the response to neoadjuvant chemotherapy: the relationship between the outcome and the clinicopathological characteristics. *Breast*. 2012;21(3):289–295.
14. Liedtke C, Mazouni C, Hess KR, et al. Response to neoadjuvant therapy and long-term survival in patients with triple-negative breast cancer. *J Clin Oncol*. 2008;26(8):1275–1281.
15. Chen IC, Lin CH, Huang CS, et al. Lack of efficacy to systemic chemotherapy for treatment of metaplastic carcinoma of the breast in the modern era. *Breast Cancer Res Treat*. 2011;130(1):345–351.
16. Carey LA, Dees EC, Sawyer L, et al. The triple negative paradox: primary tumor chemosensitivity of breast cancer subtypes. *Clin Cancer Res*. 2007;13(8):2329–2334.
17. Frampton GM, Fichtenholtz A, Otto GA, et al. Development and validation of a clinical cancer genomic profiling test based on massively parallel DNA sequencing. *Nat Biotechnol*. 2013;31(11):1023–1031.
18. Simon R, Roychowdhury S. Implementing personalized cancer genomics in clinical trials. *Nat Rev Drug Discov*. 2013;12(5):358–369.
19. Kamalakaran S, Varadan V, Janevski A, et al. Translating next generation sequencing to practice: opportunities and necessary steps. *Mol Oncol*. 2013;7(4):743–755.
20. Weigelt B, Kreike B, Reis-Filho JS. Metaplastic breast carcinomas are basal-like breast cancers: a genomic profiling analysis. *Breast Cancer Res Treat*. 2009;117(2):273–280.
21. Cooper CL, Karim RZ, Selinger C, Carmalt H, Lee CS, O'Toole SA. Molecular alterations in metaplastic breast carcinoma. *J Clin Pathol*. 2013;66(6):522–528.
22. Horlings HM, Weigelt B, Anderson EM, et al. Genomic profiling of histological special types of breast cancer. *Breast Cancer Res Treat*. 2013 Nov;142(2):257–269.
23. Prat A, Parker JS, Karginova O, et al. Phenotypic and molecular characterization of the claudin-low intrinsic subtype of breast cancer. *Breast Cancer Res*. 2010;12(5):R68. doi:10.1186/bcr2635.
24. Geyer FC, Weigelt B, Natrajan R, et al. Molecular analysis reveals a genetic basis for the phenotypic diversity of metaplastic breast carcinomas. *J Pathol*. 2010;220(5):562–573.
25. Chin K, DeVries S, Fridlyand J, et al. Genomic and transcriptional aberrations linked to breast cancer pathophysiologies. *Cancer Cell*. 2006;10(6):529–541.
26. Lien HC, Lin CW, Mao TL, Kuo SH, Hsiao CH, Huang CS. p53 overexpression and mutation in metaplastic carcinoma of the breast: genetic evidence for a monoclonal origin of both the carcinomatous and the heterogeneous sarcomatous components. *J Pathol*. 2004;204(2):131–139.
27. Reis-Filho JS, Pinheiro C, Lambros MB, et al. EGFR amplification and lack of activating mutations in metaplastic breast carcinomas. *J Pathol*. 2006;209(4):445–453.
28. Gilbert JA, Goetz MP, Reynolds CA, et al. Molecular analysis of metaplastic breast carcinoma: high EGFR copy number via aneusomy. *Mol Cancer Ther*. 2008;7(4):944–951.
29. Hayes MJ, Thomas D, Emmons A, Giordano TJ, Kleer CG. Genetic changes of Wnt pathway genes are common events in metaplastic carcinomas of the breast. *Clin Cancer Res*. 2008;14(13):4038–4044.
30. Lin SY, Xia W, Wang JC, et al. β-catenin, a novel prognostic marker for breast cancer: its roles in cyclin D1 expression and cancer progression. *Proc Natl Acad Sci U S A*. 2000;97(8):4262–4266.
31. Geyer FC, Lacroix-Triki M, Savage K, et al. β-catenin pathway activation in breast cancer is associated with triple-negative phenotype but not with CTNNB1 mutation. *Mod Pathol*. 2011;24(2):209–231.
32. Lien HC, Hsiao YH, Lin YS, et al. Molecular signatures of metaplastic carcinoma of the breast by large-scale transcriptional profiling: identification of genes potentially related to epithelial-mesenchymal transition. *Oncogene*. 2007;26(57):7859–7871.
33. Sarrió D, Rodriguez-Pinalla SM, Hardisson D, et al. Epithelial- mesenchymal transition in breast cancer relates to the basal-like phenotype. *Cancer Res*. 2008;68(4):989–997.
34. Tomaskovic-Crook E, Thompson EW, Thiery JP. Epithelial to mesenchymal transition and breast cancer. *Breast Cancer Res*. 2009;11(6):213. doi:10.1186/bcr2416.
35. Hennessy BT, Gonzalez-Angulo AM, Stemke-Hale K, et al. Characterization of a naturally occurring breast cancer subset enriched in epithelial-to-mesenchymal transition and stem cell characteristics. *Cancer Res*. 2009;69(10):4116–4124.
36. Taube JH, Herschkowitz JI, Komurov K, et al. Core epithelial-to-mesenchymal transition interactome gene-expression signature is associated with claudin-low and metaplastic breast cancer subtypes. *Proc Natl Acad Sci U S A*. 2010;107(44):15449–15454.
37. Zhang Y, Toy KA, Kleer CG. Metaplastic breast carcinomas are enriched in markers of tumor-initiating cells and epithelial to mesenchymal transition. *Mod Pathol*. 2011;25(2):178–84.
38. Moody SE, Perez D, Pan TC, et al. The transcriptional repressor Snail promotes mammary tumor recurrence. *Cancer Cell*. 2005;8(3):197–209.
39. Gwin K, Buell-Gutbrod R, Tretiakova M, Montag A. Epithelial-to-mesenchymal transition in metaplastic breast carcinomas with chondroid differentiation: expression of the E-cadherin repressor Snail. *Appl Immunohistochem Mol Morphol*. 2010;18(6):526–531.
40. Nassar A, Sookhan N, Santisteban M, et al. Diagnostic utility of snail in metaplastic breast carcinoma. *Diagn Pathol*. 2010;5:76. doi:0.1186/1746-1596-5-76.
41. Miller VA, Ross JS, Wang K, et al. Use of next-generation sequencing (NGS) to identify actionable genomic alterations (GA) in diverse solid tumor types: The Foundation Medicine (FMI) experience with 2,200+ clinical samples [abstract]. *J Clin Oncol*. 2013;31(15):11020.
42. An HX, Beckmann MW, Reifenberger G, Bender HG, Niederacher D. Gene amplification and overexpression of CDK4 in sporadic breast carcinomas is associated with high tumor cell proliferation. *Am J Pathol*. 1999;154(1):113–118.
43. Węsierska-Gądek J, Kramer MP. The impact of multi-targeted cyclin-dependent kinase inhibition in breast cancer cells: clinical implications. *Expert Opin Investig Drugs*. 2011;20(12):1611–1628.
44. Roberts PJ, Bisi JE, Strum JC, et al. Multiple roles of cyclin-dependent kinase 4/6 inhibitors in cancer therapy. *J Natl Cancer Inst*. 2012;104(6):476–487.
45. Pitts TM, Davis SL, Eckhardt SG, Bradshaw-Pierce EL. Targeting nuclear kinases in cancer: development of cell cycle kinase inhibitors. *Pharmacol Ther*. 2014;142(2):258–269.
46. Flaherty KT, Lorusso PM, Demichele A, et al. Phase I, dose-escalation trial of the oral cyclin-dependent kinase 4/6 inhibitor PD 0332991, administered using a 21-day schedule in patients with advanced cancer. *Clin Cancer Res*. 2012;18(2):568–576.
47. Engelman JA. Targeting PI3K signalling in cancer: opportunities, challenges and limitations. *Nat Rev Cancer*. 2009;9(8):550–562.
48. Isakoff SJ, Engelman JA, Irie HY, et al. Breast cancer-associated PIK3CA mutations are oncogenic in mammary epithelial cells. *Cancer Res*. 2005;65(23):10992–11000.
49. Stemke-Hale K, Gonzalez-Angulo AM, Lluch A, et al. An integrative genomic and proteomic analysis of PIK3CA, PTEN, and AKT mutations in breast cancer. *Cancer Res*. 2008;68(15):6084–6091.
50. Forbes SA, Bindal N, Bamford S, et al. COSMIC: mining complete cancer genomes in the Catalogue of Somatic Mutations in Cancer. *Nucleic Acids Res*. 2011;39(database issue):D945–D950. doi:10.1093/nar/gkq929.
51. Balko JM, Giltnane JM, Wang K, et al. Molecular profiling of the residual disease of triple-negative breast cancers after neoadjuvant chemotherapy identifies actionable therapeutic targets. *Cancer Discov*. 2014;4(2):232–245.
52. Kalinsky K, Jacks LM, Heguy A, et al. PIK3CA mutation associates with improved outcome in breast cancer. *Clin Cancer Res*. 2009;15(16):5049–5059.
53. Courtney KD, Corcoran RB, Engelman JA. The PI3K pathway as drug target in human cancer. *J Clin Oncol*. 2010;28(6):1075–1083.
54. Baselga J. Targeting the phosphoinositide-3 (PI3) kinase pathway in breast cancer. *Oncologist*. 211;16(suppl 1):12–19.
55. Janku F, Tsimberidou AM, Garrido-Laguna I, et al. PIK3CA mutations in patients with advanced cancers treated with PI3K/AKT/mTOR axis inhibitors. *Mol Cancer Ther*. 2011;10(3):558–565.
56. Baselga J, Campone M, Piccart M, et al. Everolimus in postmenopausal hormone-receptor-positive advanced breast cancer. *N Engl J Med*. 2012;366(6):520–529.
57. Simpson L, Parsons R. PTEN: life as a tumor suppressor. *Exp Cell Res*. 2001;264(1):29–41.